REMARKS

Claims 1-5, 30-44, 63 and 66 are canceled without prejudice or disclaimer. Claims 67-87 have been added and therefore are pending in the present application. Claim 67 and 77 ar supported by claims 1 and 2 as originally filed, and page 13, lines 30-32 of the specification. Claims 68-87 are supported by the claims as originally filed.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. The Objection to Claim 63

Claim 63 is objected to because it contains non-elected subject matter. Applicants request clarification of this objection. Applicants interpreted the Office Action of September 10, 2002 as requiring an election of two species of the Markush group recited in claim 63, and not a restriction requirement.

II. The Rejection of Claims 1-5, 44, and 63 under 35 U.S.C. 112

Claims 1-5, 44, and 63 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art, that the inventor(s), at the time the application was filed, had possession of the claimed invention. Specifically, the Office stated that "Applicants provide a written description only on the use of *Bacillus* cells containing very specific conditionally essential genes" and that "it is unclear from the specification what genes can be made conditionally essential gene." This rejection is respectfully traversed.

The present invention relates to methods for producing a protein comprising culturing a bacterial cell comprising at least two copies of a gene encoding the protein, wherein said cell is produced by integrating at least one DNA construct into a non-functional conditionally essential chromosomal gene(s) of the bacterial host, wherein the DNA construct comprises a non-functional copy of the conditionally essential gene(s) and at least one copy of the gene encoding the protein located between the non-functional copy and a DNA fragment homologous to a DNA sequence located adjacent to the non-functional conditionally essential gene(s) of the chromosome; wherein a first recombination between the non-functional conditionally essential gene and the non-functional copy results in a functional conditionally essential gene(s) located on the chromosome.

The specification provides numerous examples of "conditionally ess ntial genes" and bacterial hosts for use in the methods of the present invention. *See, e.g.*, page 12, lines 3-16 and pages 13-20. Based on Applicants' disclosure, the skilled person would readily be able to identify other conditionally essential genes and bacterial host cells suitable for use in the methods of the present invention, since such genes and host cells are well known in the art. Indeed, many suitable conditionally essential genes are available by sequence in various public databases. In addition, many conditionally essential genes and bacterial hosts are available in various public depositories.

Applicants submit that the specification reasonably conveys to persons skilled in the art that the inventor had possession of the claimed methods and therefore complies with the written description requirement.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 1-5, 44 and 63 under 35 U.S.C. 112

Claims 1-5, 44 and 63 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite. The Office Action provided five grounds for this rejection.

Grounds 1, 2, 4 and 5

The Office objected to claims 1 and 2 as incomplete for allegedly omitting the essential step of the recombination event that results in the restoration of the conditionally essential gene. In addition, the Office objected to the phrases "an altered non-functional copy of the conditionally essential gene" in section (b), part (i) of claims 1 and 2, the phrase "genes of step (a)" in claim 1, and the term "for example" in claim 63.

Claims 1-5, 44 and 63 have been rewritten as claims 67-87 to address these grounds of the rejection. Applicants therefore submit that these grounds have been overcome.

Ground 3

The Office objected to the term "adjacent" in claims 1 and 2. This ground is respectfully traversed.

Applicants submit that the term "adjacent" is precisely defined on page 23, lin s 10-21 of the description, as follows:

The method of the first aspect describes the integration of a gene of interest into the chromosome of a host cell, so that the gene of interest is integrated in a position that is **adjacent** to th conditionally essential locus. The exact relative positions of the gene of interest and the locus are not of major relevance for the method, however generally speaking it is of interest to minimize the distance in basepairs separating the two, both to achieve a more stable integration, but also to minimize the integration of superfluous DNA sequence into the host cell genome.

Accordingly a preferred embodiment of the invention relates to the host cell of the third aspect, wherein the gene of interest is separated from the conditionally essential locus by no more than 1000 basepairs, preferably no more than 750 basepairs, more preferably no more than 500 basepairs, even more preferably no more than 250 basepairs, and most preferably no more than 100 basepairs.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

IV. The Rejection of Claims 1, 2 and 44 under 35 U.S.C. 102

Claims 1, 2, and 44 are rejected under 35 U.S.C. 102 as being anticipated by De Groot et al. (WO 99/32641). This rejection is respectfully traversed.

De Groot et al. disclose a one-step site-specific integration of multiple copies of a gene of interest into an endonuclease restriction-site. The DNA construct used in the methods contains multiple copies of the gene of interest surrounded by two DNA fragments homologous to part of the DNA upstream and downstream of said restriction site. The DNA construct is integrated through the action of the nuclease in combination with homologous recombination. These methods are described for use in moulds (defined as filamentous fungi), not bacterial cells.

However, De Groot et al. do not teach or suggest methods of introducing one or more copies of a gene of interest into a bacterial cell at different positions.

Moreover, De Groot et al. teach away from the methods of the present invention which employ a host cell already comprising one or more copies of the gene of interest. For example, on page 6, lines 3-5, De Groot et al. states: "*A further disadvantage of the method described is the risk that the earlier introduced desired foreign DNA is removed during a subsequent repetition of the process.*"

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 102. Applicants respectfully request reconsideration and withdrawal of the rejection.

V. Conclusi n

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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